

TRAITE DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
AUX FINS DE LA PROCEDURE EN MATIERE DE BREVETS

FORMULE INTERNATIONALE

DESTINATAIRE :

NESTEC S.A.
Service des Brevets
Avenue Nestlé 55
1800 VEVEY
Suisse

RECEPISSE EN CAS DE DEPOT INITIAL,
délivré en vertu de la règle 7.1 par
l'AUTORITE DE DEPOT INTERNATIONALE
identifiée au bas de cette page

NOM ET ADRESSE
DU DEPOSANT

I. IDENTIFICATION DU MICRO-ORGANISME

Référence d'identification donnée par le
DEPOSANT :

pLL112

Numéro d'ordre attribué par
l'AUTORITE DE DEPOT INTERNATIONALE :

I - 2089

II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE

Le micro-organisme identifié sous chiffre I était accompagné :



d'une description scientifique



d'une désignation taxonomique proposée

(Cocher ce qui convient)

III. RECEPTION ET ACCEPTATION

La présente autorité de dépôt internationale accepte le micro-organisme identifié sous
chiffre I, qu'elle a reçu le **04 NOVEMBRE 1998** (date du dépôt initial)

IV. RECEPTION D'UNE REQUETE EN CONVERSION

La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous
chiffre I le **_____** (date du dépôt initial)
et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de
Budapest le **_____** (date de réception de la requête en conversion)

V. AUTORITE DE DEPOT INTERNATIONALE

Nom :

CNCM

Collection Nationale
de Cultures de Microorganismes

Signature(s) de la (des) personne(s)
compétente(s) pour représenter l'autorité
de dépôt internationale ou de l'(des)
employé(s) autorisé(s) : **Mme Y. CERISIER**
Directeur Administratif de la CNCM

Adresse :

INSTITUT PASTEUR

28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Date : **Paris, le 14 janvier 1999**

Cerisier

¹ En cas d'application de la règle 6.4.d), cette date est la date à laquelle le statut d'autorité de dépôt internationale a été acquis.

TRAITE DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
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l'AUTORITE DE DEPOT INTERNATIONALE
identifiée au bas de cette page

NOM ET ADRESSE
DU DEPOSANT

I. IDENTIFICATION DU MICRO-ORGANISME

Référence d'identification donnée par le
DEPOSANT :

PLL115

Numéro d'ordre attribué par
l'AUTORITE DE DEPOT INTERNATIONALE :

I - 2090

II. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE

Le micro-organisme identifié sous chiffre I était accompagné :



d'une description scientifique



d'une désignation taxonomique proposée

(Cocher ce qui convient)

III. RECEPTION ET ACCEPTATION

La présente autorité de dépôt internationale accepte le micro-organisme identifié sous
chiffre I, qu'elle a reçu le **04 NOVEMBRE 1998** (date du dépôt initial)¹

IV. RECEPTION D'UNE REQUETE EN CONVERSION

La présente autorité de dépôt internationale a reçu le micro-organisme identifié sous
chiffre I le _____ (date du dépôt initial)
et a reçu une requête en conversion du dépôt initial en dépôt conforme au Traité de
Budapest le _____ (date de réception de la requête en conversion)

V. AUTORITE DE DEPOT INTERNATIONALE

Nom :

CNCM

Collection Nationale
de Cultures de Microorganismes

Adresse :

INSTITUT PASTEUR

28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Signature(s) de la (des) personne(s)
compétente(s) pour représenter l'autorité
de dépôt internationale ou de l'(des)
employé(s) autorisé(s) : **Mme Y. CERISIER**
Directeur Administratif de la CNCM

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Date : Paris, le 14 janvier 1999

¹ En cas d'application de la règle 6.4.d), cette date est la date à laquelle le statut
d'autorité de dépôt internationale a été acquis.

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INTERNATIONALE DU DEPOT DES MICRO-ORGANISMES
AUX FINS DE LA PROCEDURE EN MATIERE DE BREVETS

FORMULE INTERNATIONALE

DESTINATAIRE :

**NESTEC S.A.
Service des Brevets
Avenue Nestlé 55
1800 VEVEY
Suisse**

RECEPISSE EN CAS DE DEPOT INITIAL,
délivré en vertu de la règle 7.1 par
l'AUTORITE DE DEPOT INTERNATIONALE
identifiée au bas de cette page

NOM ET ADRESSE
DU DÉPOSANT

I. IDENTIFICATION DU MICRO-ORGANISME

Référence d'identification donnée par le
DEPOSANT :

Numéro d'ordre attribué par
L'AUTORITE DE DEPOT INTERNATIONALE :

PLL116

III. DESCRIPTION SCIENTIFIQUE ET/OU DESIGNATION TAXONOMIQUE PROPOSEE

Le micro-organisme identifié sous chiffre I était accompagné :

- d'une description scientifique
- d'une désignation taxonomique proposée

(Cocher ce qui convient)

III. RECEPTION ET ACCEPTATION

La présente autorité de dépôt internationale accepte le micro-organisme identifié sous
chiffre I, qu'elle a reçu le **04 NOVEMBRE 1998** (date du dépôt initial) 1

IV. RECEPTION D'UNE REQUETE EN CONVERSION

V. AUTORITE DE DEPOT INTERNATIONALE

Nome

CNCM

Collection Nationale
de Cultures de Microorganismes

Signature(s) de la (des) personne(s) compétente(s) pour représenter l'autorité de dépôt internationale ou de l'(des) employé(s) autorisé(s) : **Mme Y. CERISIER**

Adresse:

INSTITUT PASTEUR
28, Rue du Docteur Roux
F-75724 PARIS CEDEX 15

Date : Paris, le 14 janvier 1999

Glenis

1 En cas d'application de la règle 6.4.d), cette date est la date à laquelle le statut d'autorité de dépôt internationale a été acquis.

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected Office

Date of mailing (day/month/year) 21 February 2001 (21.02.01)	
International application No. PCT/EP00/05834	Applicant's or agent's file reference 80197/WO
International filing date (day/month/year) 23 June 2000 (23.06.00)	Priority date (day/month/year) 30 June 1999 (30.06.99)
Applicant GERMOND, Jacques, Edouard et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

08 January 2001 (08.01.01)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer R. E. Stoffel Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

REC'D 12 NOV 2001
PCT

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Applicant's or agent's file reference 80197/WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/05834	International filing date (day/month/year) 23/06/2000	Priority date (day/month/year) 30/06/1999
International Patent Classification (IPC) or national classification and IPC C12N15/31		
<p>Applicant SOCIETE DES PRODUITS NESTLE S.A. et al.</p>		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 2 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 08/01/2001	Date of completion of this report 05.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Barnas, C Telephone No. +49 89 2399 7469



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/05834

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-28 as originally filed

Claims, No.:

6-15 as originally filed

1-5,16,17 as received on 04/10/2001 with letter of 04/10/2001

Drawings, sheets:

1-9 as originally filed

Sequence listing part of the description, pages:

1-7, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/05834

listing has been furnished.

4. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/05834

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1: EP-A-0 643 137 (NESTLE SA) 15 March 1995 (1995-03-15)

Arts. 33(2)(3) PCT, Novelty and Inventive Step

1. The present set of claims which refers to the lac operon of *Lactobacillus delbrueckii* subsp. *lactis* or to subregions of said operon is new over the cited prior art.
2. D1 represents the closest prior art for the subject matter of the present application. Said document discloses a DNA sequence containing gene of interest (chloramphenicol acetyltransferase) under the control of the lac promoter/operator of *L. delbrueckii bulgaricus* (p. 4, ln. 16-25 and Fig. 5). Said DNA does not contain a lac repressor protein.
3. The difference between D1 and the present application is the isolation of the lac operon of *Lactobacillus delbrueckii lactis* and its use for the expression of heterologous genes of interest. The cited prior art does not contain any indication that would prompt the skilled person to isolate said operon. The present set of claims which refers to said operon or to subregions of said operon is, therefore, inventive.

Re Item VIII

Certain observations on the international application

1. SEQ ID NO: 9 is the lac promoter/operator of *L. delbrueckii lactis*. Said DNA sequence binds to the lac repressor protein of *L. delbrueckii lactis*. The subsentence "... which retains its capability to bind ... of *L. delbrueckii*..." in claim 1 is, therefore, an undue repetition of an inherent capability of SEQ ID NO: 9. In addition, SEQ ID NO: 9 only binds to the repressor of *Lactobacillus delbrueckii lactis* not of *Lactobacillus delbrueckii bulgaricus*. The latter is, however, embraced by the term *Lactobacillus delbrueckii*. Claim 1 is, therefore, neither concise nor clear (Art. 6 PCT).
2. Claim 6 states that the DNA of the proceeding claims should be devoid of catabolite responsive elements. "Catabolite responsive elements" is however, only an

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/05834

alternative expression for "lac repressor" in the context of the present application. DNAs which are devoid of such elements/repressors are, however, already described in claim 1 ("R_y wherein Y is 0 or 1"). Claim 6, therefore, unduly describes the same subject matter which is already described in claim 1. The present set of claims is, therefore, also because of this reason not concise (Art. 6 PCT).

Amended Claims

1. A DNA sequence represented by the following general formula

$p/o - (A)_n - R_y$, or

$p/o - R_y - (A)_n$

wherein

p/o denotes the DNA sequence identified under SEQ ID No. 9 ~~or a functional variant thereof~~, which retains its capability to bind to the lac repressor protein of *Lactobacillus delbrueckii*;

A denotes a ^{heterologous} gene coding for a polypeptide of interest,

n denotes an integer of ≥ 0 ;

R denotes a gene coding for the lac repressor protein as identified under SEQ ID No. 2 ~~or a functional variant thereof~~; and

Y is 0 or 1.

2. The DNA sequence according to claim 1, wherein Y is 1.
3. The DNA sequence according to claim 1, wherein the reading frame of gene coding for the lac repressor is reversed relative to the region p/o .
4. The DNA sequence according to claim 1 wherein the gene coding for a polypeptide of interest is selected from group consisting of genes encoding enzymes, cell surface proteins, or functional peptides.
5. The DNA sequence according to claim 4, wherein the gene coding for a polypeptide of interest is selected from the group consisting of genes coding for dextranucrase, glycosyltransferase, phytase, transglutaminase, peptidase, phenylalanine ammonia lyase, protease, cell surface antigens, bacteriocins, hormones or insulin.

16. The use according to any of the claims 13 to 15, wherein expression is performed in microorganisms selected from the group consisting of lactic acid bacteria.
17. The use of a microorganism according to any of the claims 8 to 11 for the production of food products.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 80197/W0	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/05834	International filing date (day/month/year) 23/06/2000	(Earliest) Priority Date (day/month/year) 30/06/1999
Applicant SOCIETE DES PRODUITS NESTLE S.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of invention is lacking (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

5

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/05834

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C12N15/74 C07K14/335 A23C9/123

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 643 137 A (NESTLE SA) 15 March 1995 (1995-03-15)	1,4-6, 8-11, 13-17
Y	SEQ ID NO 2 page 4, line 17 - line 25 page 5, line 1 - line 13 page 20 -page 22	2,3
Y	WO 93 17117 A (LYNXVALE LTD) 2 September 1993 (1993-09-02)	2,3
A	page 1, line 23 - line 26 page 17, line 13 -page 18, line 2; figure 4 page 21, line 10 - line 24 ---	1,4, 6-11, 13-17
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

3 November 2000

09/11/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

van Klompenburg, W

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/05834

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEONG-MORGENTHALER P ET AL: "LACTOSE METABOLISM IN LACTOBACILLUS BULGARICUS: ANALYSIS OF THE PRIMARY STRUCTURE AND EXPRESSION OF THE GENES INVOLVED" JOURNAL OF BACTERIOLOGY, vol. 173, no. 6, 1 March 1991 (1991-03-01), pages 1951-1957, XP000749822 ISSN: 0021-9193 page 1953; figure 3	1,4,6, 8-11, 13-16
X	US 5 676 985 A (WU YALING ET AL) 14 October 1997 (1997-10-14) column 2, line 52 - line 58 column 3, line 5 - line 12 column 5, line 27 - line 35 column 7, line 33 -column 8, line 10; claim 5	1,4,6, 8-11, 13-17
A	DUBENDORFF J W ET AL: "CONTROLLING BASAL EXPRESSION IN AN INDUCIBLE T7 EXPRESSION SYSTEM BY BLOCKING THE TARGET T7 PROMOTER WITH LAC REPRESSOR" JOURNAL OF MOLECULAR BIOLOGY, vol. 219, no. 1, 1 January 1991 (1991-01-01), pages 45-59, XP000605448 ISSN: 0022-2836 figures 3,9	2,3,7
A	AJDIC ET AL.: "Organization and nucleotide sequence of the Streptococcus mutans galactose operon" EMBL ACC NO U21942, vol. 180, 18 March 1997 (1997-03-18), pages 137-144, XP002121893 the whole document -& AJDIC ET AL.: "Organization and nucleotide sequence of the Streptococcus mutans galactose operon" GENE, vol. 180, 1996, pages 137-144, XP002122211 figure 1	7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/05834

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0643137 A	15-03-1995	AU	678835 B	12-06-1997
		AU	7024494 A	09-03-1995
		BR	9403325 A	11-04-1995
		CA	2130784 A	27-02-1995
		CN	1104253 A, B	28-06-1995
		JP	3060399 B	10-07-2000
		JP	7303486 A	21-11-1995
		NZ	264299 A	25-06-1996
		US	5639644 A	17-06-1997
		ZA	9406464 A	21-04-1995
WO 9317117 A	02-09-1993	EP	0628083 A	14-12-1994
		GB	2278358 A, B	30-11-1994
		HK	1005465 A	08-01-1999
		JP	7504815 T	01-06-1995
US 5676985 A	14-10-1997	AT	179868 T	15-05-1999
		AU	711640 B	21-10-1999
		AU	3602395 A	06-05-1996
		CA	2202373 A	25-04-1996
		WO	9611586 A	25-04-1996
		DE	69509669 D	17-06-1999
		DE	69509669 T	21-10-1999
		DK	785727 T	29-11-1999
		EP	0785727 A	30-07-1997
		ES	2132713 T	16-08-1999
		GR	3030928 T	30-11-1999
		JP	10509304 T	14-09-1998